

### **AMENDMENTS TO THE SPECIFICATION**

Please replace the paragraph numbered [0004] with the following amended paragraph:

[0004] TRAF-3 gene products are signaling molecules that interact with the cytoplasmic tails of CD40 (Cheng, *et al.*, 1995; Hu, *et al.*, 1994; Sato, *et al.*, 1995), other TNF-R family members (Mosialos, *et al.*, 1995; Gedrich, *et al.*, 1996; Boucher, *et al.*, 1997; Yamamoto, *et al.*, 1998; Vanarsdale, *et al.*, 1997; Arch and Thompson, 1998; Kawamata, *et al.*, 1998) and the Epstein-Barr virus latent membrane protein, LMP1 (Mosialos, *et al.*, 1995). TRAF-3 is essential for T cell-dependent antibody production (Xu, *et al.*, 1996) and TRAF-3 splice-deletion isoforms activate NF- $\kappa$ B ~~NF- $\kappa$ B~~ (van Eyndhoven, *et al.*, 1999), which is known to be important in this process (Berberich, *et al.*, 1994; Snapper, *et al.*, 1996; Hostager, *et al.*, 1996; Hsing and Bishop, 1999; Grumont, *et al.*, 1998; Attar, *et al.*, 1998; Horwitz, *et al.*, 1999). However, the mechanisms by which TRAF-3 mediates signaling are not completely understood. Since TRAF-3 is a cytoplasmic, not a nuclear protein (Mosialos, *et al.*, 1995) and appears to lack catalytic activity, as do the cytoplasmic tails of all known mammalian TNF-R family members (Becraft, *et al.*, 1996), it is generally believed that TRAF-3 is an adapter molecule. In this regard, TRAF-3 has been found to interact with several other molecules including NF- $\kappa$ B ~~NF- $\kappa$ B~~ inducing kinase (NIK) (Song, *et al.*, 1997; Malinin, *et al.*, 1997), Apoptosis Signal-regulating Kinase 1 (ASK1) (Nishitoh, *et al.*, 1998), TRAF-5 (Pullen, *et al.*, 1998) and I-TRAF/ TANK (Rothe, *et al.*, 1996; Cheng and Baltimore, 1996). It has been suggested that such

interactions provide the means by which TRAF-3 mediates signal transduction, but it is unknown whether TRAF-3 associates with other molecules in an active signaling complex and/or shuttles between the inner surface of the cell membrane and some other subcellular location.

Please replace the paragraph numbered [0005] with the following amended paragraph:

[0005] TRAF-3 is known to serve essential, non-redundant functions, since TRAF-3 deficient mice are runted and die shortly after birth (Xu, *et al.*, 1996). Furthermore, TRAF-3 plays an essential and non-redundant role in signaling events underlying T cell-directed B cell differentiation, since lethally irradiated mice reconstituted with TRAF-3<sup>-/-</sup> lymphocytes exhibit defective T-dependent antibody formation (Xu, *et al.*, 1996). NF-κB ~~NF-6B~~ activation is relevant to this process because inhibition of NF-κB ~~NF-6B~~ activation significantly impairs T-dependent antibody formation *in vivo* (Snapper, *et al.*, 1996; Hostager, *et al.*, 1996; Hsing and Bishop, 1999; Grumont, *et al.*, 1998; Attar, *et al.*, 1998; Horwitz, *et al.*, 1999). In addition, over-expression of certain TRAF-3 splice-deletion isoforms, induces NF-κB ~~NF-6B~~ activation (van Eindhoven, *et al.*, 1999). T-dependent antibody production is also known to depend on CD40 signaling (Lederman, *et al.*, 1996), which induces NF-κB ~~NF-6B~~ activation (Berberich, *et al.*, 1994). Together, these findings suggest that TRAF-3 mediated NF-κB ~~NF-6B~~ activation plays a role in T-dependent antibody production.

Please replace the paragraph numbered [0007] with the following amended paragraph:

[0007] The TRAF-3 isoleucine zipper (or coiled-coil) domain has been shown to participate in TRAF-3 interactions with TRAF-5, the only other known TRAF that contains an isoleucine zipper (Pullen, *et al.*, 1998). The TRAF-3 Zn finger domain contains five atypical Zn fingers in full-length TRAF-3 and fewer fingers with different compositions in splice-deletion isoforms that induce NF- $\kappa$ B ~~NF-6B~~ activation (Eyndhoven, *et al.*, 1999). The TRAF-3 Zn fingers and Ring finger are required for the ability to induce NF- $\kappa$ B ~~NF-6B~~ activation in both TRAF-3 splice-variants and in TRAF-3/TRAF-5 chimeric molecules (Dadgostar and Cheng, 1988). However, the interactions between the TRAF-3 Ring and Zn fingers with other factors that regulate NF- $\kappa$ B ~~NF-6B~~ activation and translocation are not completely understood. The mechanism by which TRAF-3 gene products induce NF- $\kappa$ B ~~NF-6B~~ activation between receptor stimulation and translocation of activated NF- $\kappa$ B ~~NF-6B~~ complexes into the nucleus remain unclear.

Please replace the paragraph numbered [0008] with the following amended paragraph:

[0008] NF- $\kappa$ B ~~NF-6B~~ proteins are transcription factors that form homo- and hetero-dimeric complexes which are retained in the cytoplasm bound to I $\kappa$ B ~~I6B~~ proteins in resting cells (reviewed in (Baeuerle and Baltimore, 1996)). Certain stimuli, such as signaling by several TNF-R family members, activate the I $\kappa$ B ~~I6B~~ (IKK) complex which phosphorylates I $\kappa$ B ~~I6B~~ proteins, ultimately releasing free NF- $\kappa$ B ~~NF-6B~~ dimers with exposed nuclear localization sequences (NLSs) (DiDonato, *et al.*, 1997; Woronicz, *et al.*, 1997; Mercurio, *et al.*, 1997). Like other NLS containing molecules, the freed NF- $\kappa$ B ~~NF-6B~~ complexes associate with the

karyopherin- $\alpha$  (importin- $\alpha$ ) cytoplasmic NLS receptor (Nadler, *et al.*, 1997). The karyopherin- $\alpha$ / NF- $\kappa$ B ~~NF- $\kappa$ B~~ complex is targeted to the nuclear pore by association with karyopherin- $\beta$  (Gorlich, *et al.*, 1995) which mediates interaction of the complex with p62 nucleoporin (p62) (Rexach and Blobel, 1995; Finlay, *et al.*, 1991; Percipalle, *et al.*, 1997). The p62 C-terminal domain binds karyopherin- $\beta$  (Percipalle, *et al.*, 1997) and an N-terminal domain binds p10/NTF2, an accessory factor that is required for the docked import complex to undergo translocation through the pore (Paschal and Gerace, 1995; Torgerson, *et al.*, 1998). After translocation, NF- $\kappa$ B ~~NF- $\kappa$ B~~ complexes bind to genomic regulatory sequences and activate transcription of target genes.

Please replace the paragraph numbered [0009] with the following amended paragraph:

[0009] Although it was known that NF- $\kappa$ B ~~NF- $\kappa$ B~~ activation and translocation across a nuclear membrane were relevant to the role TRAF-3 plays in signaling events underlying T cell-directed B cell differentiation, it was not known, expected or realized that particular fragments of the p62 nucleoporin polypeptide affect ~~effect~~ this process in ways that the complete p62 polypeptide does not. Thus, the polypeptides of the present invention regulate T cell-dependent antibody production against antigens and provide new immunotherapies and treatments.

Please replace the paragraph numbered [0011] with the following amended paragraph:

[0011] One embodiment of the present invention provides an isolated polypeptide derived from the p62 nucleoporin protein, p62(1-393) ~~p62(1-392)~~, of the structure of formula I and salts thereof:

MSGFNFGGTG APTGGFTFGT AKTATTTTPAT GFSFSTSGTG  
GFNFGAPFQP ATSTPSTGLF SLATQTPATQ TTGFTFGTAT LASGGTGFSL  
GIGASKLNLS NTAATPAMAN PSGFGLGSSN LTNAISSTVT SSQGTAPTGF  
VFGPSTTSVA PATTSGGFSF TGGSTAQPSG FNIGSAGNSA QPTAPATLPF  
TPATPAATTA GATQPAAPTP TATITSTGPS LFASIATAPT SSATTGLSLC  
TPVTTAGAPT AGTQGFSLKA PGAASGTSTT TSTAATATAT TTTSSSTTGF  
ALNLKPLAPA GIPSNTAAAV TAPPGPGAAA GAAASSAMTY AQLESLINKW  
SLELEDQERH FLQQATQVNA WDRTLIENG KITS�HREVE KVKLDQKRLD  
QEL (SEQ ID NO:1).

Please replace the paragraph numbered [0013] with the following amended paragraph:

[0013] The present invention also provides an isolated polypeptide derived from the p62 nucleoporin, p62(336-522), of the structure of formula II and salts thereof:

LINKWSLELE DQERHFLQQA TQVNAWDRTL IENGKITSL  
HREVEKVKLD QKRLDQELDF ILSQQKELED LLSPLEELVK EQRATYLYQH  
ADEERQKTYK LAENIDAQLK RMAQDLKDII EHLNTSGAPA DTSDPLQQIC  
KILNAHMDSL QWIDQNSALL QRKVEEVTKV CVGRRKEQER SFRITFD  
(SEQ ID NO:2).

Please replace the paragraph numbered [0015] with the following amended paragraph:

[0015] The invention also provides pharmaceutical compositions comprising a therapeutically effective amount of a polypeptide of the invention and a pharmaceutically acceptable carrier. Methods of inhibiting translocation of activated NF- $\kappa$ B ~~NF-6B~~ and enhancing activation of NF- $\kappa$ B ~~NF-6B~~ in a mammal by administering a pharmaceutical composition of the invention, is also provided. The invention further provides methods of providing p62(1-393) ~~p62(1-392)~~ or p62(336-522) to cells comprising contacting polypeptides with cells that have been treated to absorb exogenous polypeptides so that the cells contain the polypeptide of the invention.

Please replace the paragraph numbered [0019] with the following amended paragraph:

[0019] FIG. 3 illustrates the results of an analysis of TRAF-3:p62 association in 293T cells. Cell lysates from 293T cells transiently transfected with the indicated epitope-tagged expression vectors were immunoprecipitated with either anti-X-press or anti-HA monoclonal antibodies. Detected proteins are labeled at the left and IgG heavy and light chains are labeled at the right. The asterisk ~~asterix~~ indicates a proteolytic fragment of His-TRAF-3 which was observed when p62(336-522) was co-expressed.

Please replace the paragraph numbered [0020] with the following amended paragraph:

[0020] FIG. 4 illustrates the results of the effects of TRAF-3 and p62 on RelA(Re1A) ~~Re1A(Re1A)~~ induced NF- $\kappa$ B ~~NF-6B~~ activation in mammalian cells. The white bars represent the activity of cultures co-transfected with empty expression vector, while grey bars represent the activity of cultures co-transfected with 100 ng of pCDNA3/p65(RelA)(~~Re1A~~). Empty pCEP4 was used to normalize DNA content of all samples. *Renilla* luciferase activity was used to scale Firefly luciferase activity for transfection efficiency. Results are normalized to the activity of cultures transfected with empty pCEP4 without RelA ~~Re1A~~ co-transfection. Error bars represent standard deviation of the mean of triplicate cultures. These data are representative of 3 independent experiments.

Please replace the paragraph numbered [0021] with the following amended paragraph:

[0021] FIG. 5 illustrates the results of the effects of TRAF-3 and p62 on CD40 induced NF- $\kappa$ B ~~NF-6B~~ activation in 293T cells. 293T cells were transiently transfected with 3 $\mu$ g ~~3ug~~ of the indicated expression vectors, 300 ng of the PRDIIx4 Luc NF- $\kappa$ B ~~NF-6B~~ reporter construct and 75 ng of pRLtk. White bars represent the activity of cultures co-transfected with empty expression vector and grey bars indicate the activity of cultures co-transfected with 500 ng of pCEP4/CD40 to activate NF- $\kappa$ B ~~NF-6B~~. Empty pCEP4 was used to normalize DNA content of all samples. *Renilla* luciferase activity was used to scale Firefly luciferase activity for transfection efficiency. Results are normalized to the activity of cultures transfected with empty pCEP4 without CD40 co-transfection. Error

bars represent standard deviation of the mean of triplicate cultures. These data are representative of 3 independent experiments.

Please replace the paragraph numbered [0026] with the following amended paragraph:

[0026] TRAF, TNF receptor-associated factor; NF- $\kappa$ B ~~NF-6B~~, Nuclear factor- $\kappa$ B ~~factor-6B~~; TNF-R, Tumor Necrosis Factor Receptor; NIK, NF- $\kappa$ B ~~NF-6B~~ -including kinase; I- $\kappa$ B ~~I-6B~~, Inhibitor of NF- $\kappa$ B ~~NF-6B~~; IKK, I- $\kappa$ B ~~I-6B~~ Kinase; N-terminal; amino-terminal; C-terminal, carboxy-terminal; p62, p62 nucleoporin; NLS, nuclear localization sequence; ORF, open reading frame; RT, reverse transcriptase; AD, Activation Domain; BD, DNA Binding Domain,  $\beta$ -gal,  $\beta$ -galactosidase; His, histidine; aa, amino acid; HA-tagged, hemagglutinin epitope tagged fusion construct; TBST, Tris-buffered saline with 0.1% Tween-20; IMDM Iscove's Modified Dulbecco's Media.

Please replace the paragraph numbered [0031] with the following amended paragraph:

[0031] The phrases "NF- $\kappa$ B activation" "~~NF-6B activation~~" or "activated NF- $\kappa$ B" "~~activated NF-6B~~" as used herein, refer to a NF- $\kappa$ B ~~NF-6B~~ molecule that dissociates from I- $\kappa$ B ~~I-6B~~, which is a complex that masks the NF- $\kappa$ B ~~NF-6B~~ NLS. Signaling by cell surface receptors leads to the dissociation of I- $\kappa$ B ~~I-6B~~ and the liberation of NF- $\kappa$ B ~~NF-6B~~ with an exposed NLS. It is this form of NF- $\kappa$ B ~~NF-6B~~, having an exposed NLS, that is referred to herein as the activated form of NF- $\kappa$ B ~~NF-6B~~.



Please replace the paragraph numbered [0038]<sup>1</sup> with the following amended paragraph:

[0038] In one embodiment, the present invention provides polypeptides derived from the p62 nucleoporin protein that inhibit the translocation of NF- $\kappa$ B ~~NF-6B~~ in ways that the complete p62 nucleoporin protein does not. In particular, this invention provides for an isolated polypeptide derived from the p62 nucleoporin protein, p62(1-393) ~~p62(1-392)~~, that inhibits the translocation of activated NF- $\kappa$ B ~~NF-6B~~ across a nucleic membrane. The amino acid sequence of p62(1-393) ~~p62(1-392)~~ is set forth as formula I in SEQ ID NO:1.

Please replace the paragraph numbered [0039] with the following amended paragraph:

[0039] There are several domains of the p62 protein, each of which have different structures and sometimes different functions. For example, the p62 C-terminal coiled-coil domain and the TRAF-3 Zn finger and coiled-coil domains are important in mediating their interaction. The interaction of p62 with TRAF-3 is specific, since p62 does not interact with TRAF-2, -4, -5 or -6. Over expression of p62(1-393) ~~p62(1-392)~~, which contains the p10/NT2 binding domain but not

---

<sup>1</sup> We note that the paragraph numbering in the application as published differs from the application as filed. In particular, it appears as if the PTO assigned paragraph no. 27 to the heading "Single and Three Letter Abbreviations for Amino Acids," paragraph no. 114 to the heading "a. Plasmid and constructs," paragraph no. 118 to the heading "b. Yeast Two-Hybrid Screening for TRAF-3 Interacting Proteins," paragraph no. 120 to the heading "c. Characterization of p62/TRAF Protein Interactions," paragraph no. 122 to the heading "d. Tissue Culture and Transient Transfection for Protein Expression," paragraph no. 124 to the heading "e. Protein Analysis," paragraph no. 128 to the heading "f. Luciferase Reporter Assays," paragraph no. 130 to the heading "a. Yeast Two-Hybrid Screen for TRAF-3 Interacting Molecules," paragraph no. 135 to the heading "b. Interactions of TRAF-3 and p62 in Mammalian Cells," paragraph no. 137 to the heading "c. Effects of p62 Fragments on RelA Translocation," and paragraph no. 139 to the heading "d. Effects of p62 Fragments on Inducing NF-KB Activation," whereas the application as filed did not assign a paragraph number to these headings. For purposes of this Response, we adopt the paragraph numbering scheme used by the PTO.

the TRAF-3 binding domain, inhibits RelA ~~Re1A~~-induced reporter activity, and inhibits NF- $\kappa$ B ~~NF-6B~~ translocation. Inhibiting NF- $\kappa$ B ~~NF-6B~~ translocation prevents NF- $\kappa$ B ~~NF-6B~~ from activating the gene normally expressed when NF- $\kappa$ B ~~NF-6B~~ binds to a regulatory region of the target gene. Thus, inhibiting NF- $\kappa$ B ~~NF-6B~~ translocation, in effect ~~affect~~, limits, if not completely blocks the transcription of genes that are normally expressed by the binding of NF- $\kappa$ B ~~NF-6B~~ to a regulatory region of a gene.

Please replace the paragraph numbered [0040] with the following amended paragraph:

[0040] Another embodiment of the present invention provides isolated polypeptides derived from the p62 nucleoporin protein that enhance the activation of NF- $\kappa$ B ~~NF-6B~~ in ways that the complete p62 nucleoporin protein does not. In particular, this invention provides for an isolated polypeptide derived from the p62 nucleoporin protein, p62(336-522), that enhances the activation of NF- $\kappa$ B ~~NF-6B~~ without affecting translocation of activated NF- $\kappa$ B ~~NF-6B~~. For example, over expression of p62(336-522), a TRAF-3 binding fragment, enhances NF- $\kappa$ B ~~NF-6B~~ activation and augments CD40-induced NF- $\kappa$ B ~~NF-6B~~ reporter gene activity, but has no effect on RelA ~~Re1A~~-induced reporter activity. This indicates that the p62(336-522) enhances the activation of NF- $\kappa$ B ~~NF-6B~~ and does not affect the translocation of activated NF- $\kappa$ B ~~NF-6B~~. The amino acid sequence of p62(336-522) is set forth as formula II in SEQ ID NO:2.

Please replace the paragraph numbered [0046] with the following amended paragraph:

[0046] After cleavage and deprotection ~~deprotection~~, compounds of the present invention are purified. For example, gel filtration chromatography and reverse-phase column/HPLC system can be used to purify full length compounds from fragments thereof. The amino acid sequences of the polypeptides produced may be confirmed and identified using standard amino acid analysis, as well as manual and automated Edman degradation and determination of each amino acid. High Pressure Liquid Chromatography (HPLC) analysis and mass spectrometry may also be used to verify the compounds produced.

Please replace the paragraph numbered [0047] with the following amended paragraph:

[0047] Computer modeling may be used to design "variants" of fragments p62(1-393) ~~p62(1-392)~~ and p62(336-522) based on their preferred structural and functional properties. Polypeptide sequences are analyzed for predicted secondary structure, hydrophobic moment, and amphipathicity. Some computer programs available include Eisenberg Algorithm (Eisenberg et al. Biopolymers 27: 171-177, 1996) for helical structure; Genetics Computer Group (Madison, Wisc.) for secondary structure, hydrophobic moment and amphipathizing and Eisenberg et al., Proc. Natl. Sci. USA 4 ed., 81:140-144 (1984) for hydrophobic moment.

Please replace the paragraph numbered [0049] with the following amended paragraph:

[0049] Other conservative substitutions of amino acids in the p62(1-393) ~~p62(1-392)~~ and p62(336-522) fragments can be taken from Table A to produce sequences that are at least 80%, 85% or 90% identical over their entire length to either the p62(1-393) ~~p62(1-392)~~ or the p62(336-522) polypeptide. Additional possible substitutions are described by Dayhoff in the Atlas of Protein Sequence and Structure (1988).

Please replace the paragraph numbered [0051] with the following amended paragraph:

[0051] Variants with amino acid substitutions which are less conservative may also result in desired derivatives that are at least 80%, 85%, 90% identical over the entire length of the p62(1-393) ~~p62(1-392)~~ and the p62(336-522) polypeptides, e.g., by causing changes in charge, conformation and other biological properties. Such substitutions would include for example, substitution of a hydrophilic residue for a hydrophobic residue, substitution of a cysteine or a proline for another residue, substitution of a residue having a small side chain for a residue having a bulky side chain or substitution of a residue having a net negative charge. When the result of a given substitution cannot be predicted with certainty, the derivatives may be readily assayed according to the methods disclosed herein to determine the presence or absence of the desired characteristics.

Please replace the paragraph numbered [0054] with the following amended paragraph:

[0054] In order to express a biologically active p62(1-393) ~~p62(1-392)~~ or p62(336-522) or variants thereof, the nucleotide sequences encoding these polypeptides may be inserted into an appropriate expression vector, i.e., a vector which contains the necessary elements for the transcription and translation of the inserted coding sequence.

Please replace the paragraph numbered [0058] with the following amended paragraph:

[0058] In bacterial systems, a number of expression vectors may be selected depending upon the use intended for the polypeptides of the invention. For example, when large quantities of these polypeptides are needed to quickly enhance the activation of NF- $\kappa$ B ~~NF-6B~~ or to quickly increase the translocation of NF- $\kappa$ B ~~NF-6B~~ vectors which direct high level expression of fusion proteins that are readily purified may be used. Such vectors include, but are not limited to, multifunctional E.coli cloning and expression vectors such as BLUESCRIPT (Stratagene), in which the sequence encoding NHLP may be ligated into the vector in frame with sequences for the amino-terminal Met and the subsequent 7 residues of  $\beta$ -galactosidase ~~beta-galactosidase~~ so that a hybrid protein is produced, and pIN vectors. (See, e.g., Van Heeke, G. and S.M. Schuster (1989), J. Biol Chem. 264: 5503-5509.) pGEX vectors (Pharmacia Biotech, Uppsala, Sweden) may also be used to express foreign polypeptides as fusion proteins with glutathione S-transferase (GST). In general, such fusion proteins are

soluble and can easily be purified from lysed cells by absorption to glutathione-agarose beads followed by elution in the presence of free glutathione. Proteins made in such systems may be designed to include heparin, thrombin, or factor XA protease cleavage sites so that the cloned polypeptide of interest can be released from the GST moiety at will.

Please replace the paragraph numbered [0063] with the following amended paragraph:

[0063] Human artificial chromosomes (HACs) ~~(Has)~~ may also be employed to deliver larger fragments of DNA than can be contained and expressed in a plasmid. (HACs) ~~(Has)~~ of about 6 kb to 10Mb are constructed and delivered via conventional delivery methods (liposomes, polycationic amino polymers, or vesicles) for therapeutic purposes.

Please replace the paragraph numbered [0064] with the following amended paragraph:

[0064] Specific initiation signals may also be used to achieve more efficient translation of sequences encoding p62(1-393) ~~p62(1-392)~~, p62(336-522) or variants thereof. Such signals include the ATG initiation codon and adjacent sequences. In cases where sequences encoding the polypeptides and its initiation codon and upstream sequences are inserted into the appropriate expression vector, no additional transcriptional or translational control signals may be needed. However, in cases where only coding sequence, or a fragment thereof, is inserted, exogenous translational control signals including the ATG

initiation codon should be provided. Furthermore, the initiation codon should be in the correct reading frame to ensure translation of the entire insert. Exogenous translational elements and initiation codons may be natural or synthetic. The efficiency of expression may be enhanced by the inclusion of enhancers appropriate for the particular cell system used ~~sued~~. (See, e.g., Scharf, D. et al. (1994) Results Probl. Cell Differ. 20: 125-162.)

Please replace the paragraph numbered [0067] with the following amended paragraph:

[0067] Any number of selection systems may be used to recover transformed cell lines. These include, but are not limited to, the herpes simplex virus thymidine kinase genes and adenine phosphoribosyltransferase genes, which can be employed in tk or apr ~~tk-sup~~ or ~~apr-sup~~ cells, respectively. (See, e.g., Wigler ~~wigler~~, M. et al. (1997) Cell 11: 223-232; and Lowy, I. et al. (1980) Cell 22: 817-823). Also, antimetabolite, antibiotic, or herbicide resistance can be used as the basis for selection. For example, dhfr confers resistance to methotrexate; npt confers resistance to the aminoglycosides neomycin and G-418; and als or pat confer resistance to chlorsulfuron and phosphinotricin acetyltransferase, respectively. (See, e.g., Wigler, M. et al. (1980) Proc. Natl. Acad. Sci. 77: 3567-3570; Colbere-Garapin, F. et al (1981) J. Mol. Biol. 150: 1-14; and Murry, supra.) Additional selectable genes have been described, e.g., trpB, which allows cells to utilize indole in place of tryptophan, or hisD, which allows cells to utilize hisinol in place of histidine. (See, e.g., Hartman, S. C. and R.C. Mulligan (1988) Proc. Natl. Acad. Sci. 85: 8047-8051.) Visible markers, e.g.,

anthocyanins, beta glucuronidase and its substrate GUS, luciferase and its substrate luciferin may be used. Green fluorescent proteins (GFP) (Clontech, Palo Alto, Calif.) can also be used. These markers can be used not only to identify transformants, but also to quantify the amount of transient or stable protein expression attributable to a specific vector system. (See, e.g., Rhodes, Calif. et al. (1995) methods Mol. Biol. 55: 121-131.)

Please replace the paragraph numbered [0068] with the following amended paragraph:

[0068] Although the presence/absence of marker gene expression suggests that the gene of interest is also present, the presence and expression of the gene may need to be confirmed. For example, if the polynucleotide sequence encoding p62(1-393) ~~p62(1-392)~~ is inserted within a marker gene sequence, transformed cells containing the polynucleotide sequences encoding p62(1-393) ~~p62(1-392)~~ can be identified by the absence of marker gene function. Alternatively, a marker gene can be placed in tandem with a sequence encoding p62(1-393) ~~p62(1-392)~~ under the control of a single promoter. Expression of the marker gene in response to induction or selection usually indicates expression of the tandem gene as well.

Please replace the paragraph numbered [0069] with the following amended paragraph:

[0069] Alternatively, host cells which contain the nucleic acid sequence encoding p62(1-393) ~~p62(1-392)~~ and express p62(1-393) ~~p62(1-392)~~ may be



identified by a variety of procedures known to those of skill in the art. These procedures include, but are not limited to, DNA-DNA or DNA-RNA hybridization and protein bioassay or immunoassay techniques which include membrane, solution, or chip based technologies for the detection and/or quantification of nucleic acid or protein sequences.

Please replace the paragraph numbered [0071] with the following amended paragraph:

[0071] A variety of protocols for detecting and measuring the expression of polypeptides of the invention, using either polyclonal or monoclonal antibodies specific for the protein, are known in the art. Examples of such techniques include enzyme-linked immunosorbent assays (ELISAs), radioimmunoassays (RIAs), and fluorescence activated cell sorting (FACs). A two-site, monoclonal-based immunoassay utilizing monoclonal antibodies reactive to two non-interfering epitopes on p62(1-393) ~~p62(1-392)~~, p62(336-522) or variants thereof is preferred, but a competitive binding assay may be employed. These and other assays are well described in the art. (See, e.g., Hampton, R. et al. (1990) Serological Methods, a Laboratory manual, ASP Press, St. Paul, Minn., Section IV; and Madden, DE et al. (1983) J. Exp. Med. 158: 1211-1216, which are herein incorporated by reference).

Please replace the paragraph numbered [0075] with the following amended paragraph:

[0075] The polypeptides of the present invention and/or salts thereof may also be formulated with pharmaceutically acceptable carriers to provide pharmaceutical compositions. Such a pharmaceutical composition may contain an enhancing NF- $\kappa$ B ~~NF-6B~~ activating effective amount of the p62(1-393) ~~p62(1-392)~~ polypeptide or one or more of the p62(1-393) ~~p62(1-392)~~ polypeptide variants or pharmaceutical acceptable salts thereof. An enhancing NF- $\kappa$ B ~~NF-6B~~ activating effective amount is an amount of the p62(1-393) ~~p62(1-392)~~ polypeptide or one or more of the p62(1-393) ~~p62(1-392)~~ polypeptide variants that is sufficient to enhance the amount of activated NF- $\kappa$ B ~~NF-6B~~ in an individual host cell as compared to the amount of NF- $\kappa$ B ~~NF-6B~~ that is activated in a cell that is not subjected to any amount of the p62(1-393) ~~p62(1-392)~~ or one or more of the p62(1-393) ~~p62(1-392)~~ polypeptide variants. The amount of NF- $\kappa$ B ~~NF-6B~~ activated in a cell can be measured by measuring the amount of free I $\kappa$ B ~~I6B~~ in the cell. To measure the amount of Free I $\kappa$ B ~~I6B~~, the I $\kappa$ B ~~I6B~~ can be tagged with a marker that is detectable once it dissociates from the NF- $\kappa$ B ~~NF-6B~~ molecule.

Please replace the paragraph numbered [0076] with the following amended paragraph:

[0076] Other pharmaceutical compositions may contain a NF- $\kappa$ B ~~NF-6B~~ translocation inhibiting amount of the p62(336-522) ~~p62(366-522)~~ polypeptide or one or more of the p62(336-522) ~~p62(366-522)~~ polypeptide variants of the polypeptides of the present invention or pharmaceutical acceptable salts thereof. An NF- $\kappa$ B ~~NF-6B~~ translocation inhibiting effective amount is an amount of the

p62(336-522) ~~p62(366-522)~~ polypeptide or one or more of the p62(336-522) ~~p62(366-522)~~ variants that is sufficient to inhibit the translocation of NF- $\kappa$ B ~~NF-6B~~ across a nuclear membrane in an individual host cell as compared to the amount of NF- $\kappa$ B ~~NF-6B~~ translocated across a nuclear membrane of a cell that is not subjected to any amount of the p62(336-522) ~~p62(366-522)~~ polypeptide or one or more of the p62(336-522) ~~p62(366-522)~~ polypeptide variants. The amount of NF- $\kappa$ B ~~NF-6B~~ translocated across a nuclear membrane of a cell can be observed by tagging the NF- $\kappa$ B ~~NF-6B~~ with a marker and detecting the marker.

Please replace the paragraph numbered [0093] with the following amended paragraph:

[0093] Pharmaceutical compositions containing the p62(1-393) ~~p62(1-392)~~ polypeptide or variants thereof may be administered to a mammal to increase the amount of activated NF- $\kappa$ B ~~NF-6B~~ in the mammal. This method of administration comprises, administering to the mammal a pharmaceutical composition comprising, in combination with a pharmaceutical acceptable carrier, a NF- $\kappa$ B ~~NF-6B~~ activating effective amount of the peptide having the formula I or variants thereof. As stated above, a NF- $\kappa$ B ~~NF-6B~~ activating effective amount is an amount of the pharmaceutical composition containing at least one polypeptide of the invention that increases the amount of activated NF- $\kappa$ B ~~NF-6B~~ in a cell that is not subjected to any amount of the polypeptide. This amount is easily quantified by measuring the amount of free NLS in the mixture.

Please replace the paragraph numbered [0094] with the following amended paragraph:

[0094] In addition, pharmaceutical compositions containing the p62(336-522) polypeptide, or variants thereof, may be administered to a mammal to inhibit the translocation of NF- $\kappa$ B ~~NF-6B~~ across a membrane in the mammal. This method of administration comprises, administering to the mammal a pharmaceutical composition comprising, in combination with a pharmaceutical acceptable carrier, a NF- $\kappa$ B ~~NF-6B~~ translocation inhibiting effective amount of the peptide having the formula III or variants thereof. As stated above, a NF- $\kappa$ B ~~NF-6B~~ translocation inhibiting effective amount is an amount of a pharmaceutical composition that contains at least one polypeptide of the invention that inhibits translocation of activated NF- $\kappa$ B ~~NF-6B~~ across a nuclear membrane as compared to the amount of activated NF- $\kappa$ B ~~NF-6B~~ that is translocated across the membrane that is not subjected to the pharmaceutical composition.

Please replace the paragraph numbered [0095] with the following amended paragraph:

[0095] In addition, an effective amount refers to that amount of the active ingredient, e.g. a p62(1-393) ~~p62(1-392)~~ or p62(336-522) polypeptide of the invention, which ameliorates the symptoms or condition. Therapeutic efficacy and toxicity may be determined by standard pharmaceutical procedures in cell cultures or with experimental animals, such as calculating the ED<sub>50</sub> ~~ED<sub>50</sub>~~ (the dose ~~does~~ therapeutically effective in 50% of the population) or the LD<sub>50</sub> ~~LD<sub>50</sub>~~ (the dose lethal in 50% of the population) statistics. The dose ratio of therapeutic

to toxic effects is the therapeutic index, and it can be expressed as the  $\frac{LD_{50}}{ED_{50}}$  ratio. Pharmaceutical compositions which exhibit large therapeutic indices are preferred. The data obtained from cell culture assays and animal studies are used to formulate a range of dosage forms for human use. The dosage contained in such compositions is preferably within a range of circulating concentrations that includes the  $ED_{50}$  with little or no toxicity. The dosage varies within this range depending upon the dosage form employed, the sensitivity of the patient, and the route of administration.

Please replace the paragraph numbered [0100] with the following amended paragraph:

[0100] The pharmaceutical compositions of the invention can be administered as the sole active pharmaceutical agent, or they can also be administered in combination with one or more molecules. Thus, although the formulations disclosed herein above are effective and relatively safe medications either inhibiting NF- $\kappa$ B translocation or activating NF- $\kappa$ B without inhibiting translocation, the possible concurrent administration of these formulations with other medications or agents to obtain beneficial results is not excluded. Such other agents may be the agents associated with gene therapy.

Please replace the paragraph numbered [0105] with the following amended paragraph:

[0105] In order to provide methods and devices for screening compounds for effects on biochemical systems, the present invention generally incorporates

model *in vitro* systems which mimic a given biochemical system *in vitro* for which effector compounds are desired. The range of systems against which compounds can be screened and for which effector compounds are desired, is extensive. For example, compounds may be screened for effects in blocking, slowing or otherwise inhibiting events associated with biochemical systems. For example, test compounds may be screened for their ability to inhibit the translocation of activated NF- $\kappa$ B ~~NF-6B~~ across the nuclear membrane of a cell which in turn inhibits the binding of the NF- $\kappa$ B ~~NF-6B~~ to the transcriptional control region of a particular gene. Thus, shortening off the gene. Alternatively, molecules that cause the dissociation of I $\kappa$ B ~~I6B~~ from NF- $\kappa$ B ~~NF-6B~~ molecule may also be detected. Compounds which show promising results in these screening assay methods can then be subjected to further testing to identify effective pharmacological agents for the treatment of disease or systems of a disease.

Please replace the paragraph numbered [0106] with the following amended paragraph:

[0106] However, in some instances, high-throughput screening assays are not able to detect weak signals of interaction between the test compounds and the biochemical system being tested unless the signals are above a specific baseline level. For example, a weak activation of NF- $\kappa$ B ~~NF-6B~~ may not be detected by a detecting system unless it is above a particular baseline level because the detecting means is not sensitive enough to detect such low levels of activity. In particular, a compound capable of enhancing the activation of NF- $\kappa$ B

~~NF-6B~~ may not be detected in an assay if the level of activation is below a certain baseline level that is specific to the detecting means. Alternatively, the level of translocation of activated NF- $\kappa$ B ~~NF-6B~~ across a nuclear membrane may also be too low to detect.

Please replace the paragraph numbered [0108] with the following amended paragraph:

[0108] One or all of these polypeptides enhance the particular activity, i.e., translocation of NF- $\kappa$ B ~~NF-6B~~ across a nuclear membrane or activation of NF- $\kappa$ B ~~NF-6B~~, above a baseline level so that minor changes in activity caused by the compound being tested can be detected by the detecting system used in the high throughput screening assay. For example, a discrete amount of polypeptide of the invention listed above may activate NF- $\kappa$ B ~~NF-6B~~ above a baseline level below which the detecting mechanism of the assay is unable to detect effects by test compounds, so that activation of additional NF- $\kappa$ B ~~NF-6B~~ molecules by the test compounds is now detectable by the detecting system.

Please replace the paragraph numbered [0109] with the following amended paragraph:

[0109] Test compounds that have only a minor effect on the activation of NF- $\kappa$ B ~~NF-6B~~, e.g., the liberation of I $\kappa$ B ~~I6B~~ from the NF- $\kappa$ B ~~NF-6B~~ exposing the NLS on the NF- $\kappa$ B ~~NF-6B~~, might not be detected by a highthrough put assay that does not contain the polypeptides of the invention because the amount of I $\kappa$ B ~~I6B~~ released from NF- $\kappa$ B ~~NF-6B~~ may be below the sensitivity level of the

detecting system. The assay of the present invention uses the polypeptides of the invention to "prime" the detecting system to be more sensitive to minor changes the amount of free I $\kappa$ B directly indicating the amount of NF- $\kappa$ B ~~NF-6B~~ activation caused by the test compound. In other words, the detecting system detects the effect of the polypeptides of the invention on NF- $\kappa$ B ~~NF-6B~~ activation as a background level, and any additional effect caused by the test compound is now within the sensitivity range of the detecting system.

Please replace the paragraph numbered [0110] with the following amended paragraph:

[0110] So too is true with effects of test compounds on the activated NF- $\kappa$ B ~~NF-6B~~ translocation across a nuclear membrane. Test compounds having only a minor effect on NF- $\kappa$ B ~~NF-6B~~ translocation.

Please replace the paragraph numbered [0117] with the following amended paragraph:

[0117] Full-length p62 was amplified by RT-PCR from Jurkat T-cell mRNA and TA cloned into pCR2.1. Deletion mutants of p62 were generated by subcloning of restriction fragments of p62 obtained in several yeast two-hybrid clones into the pGAD424 activation domain vector (Clontech Laboratories). An oligonucleotide linker encoding an HA tag and stop codon was ligated into pCR2.1/p62 at a unique SacI site to generate a truncated p62(aa 1-393) ~~p62(aa 1-392)~~ construct with a C-terminal HA tag. Full-length p62, HA-tagged p62(1-393) ~~p62(1-392)~~, and HA-tagged p62(336-522) were subcloned into pCEP4 for



mammalian expression. PRDIIx4 Luc was generated by subcloning the NF-κB ~~NF-6B~~ sites and interferon beta promoter from PRDIIx4 CAT into the pGL3 Enhance plasmid (Promega, Madison, WI). PRLtk (Promega) which constitutively expresses *Renilla* Luciferase was used to control for transfection efficiency in reporter assays. DNA for mammalian transfection was prepared with Maxiprep and Megaprep columns (Qiagen, Valencia, CA).

Please replace the paragraph numbered [0138] with the following amended paragraph:

[0138] To study the functional effects of p62 on NF-κB ~~NF-6B~~ activation and translocation, the first series of experiments evaluated the effects of over-expressing p62 fragments on the translocation of activated NF-κB ~~NF-6B~~. Over-expression of RelA results in excess free RelA which is able to dimerize, translocate to the nucleus, and activate transcription of NF-κB ~~NF-6B~~ responsive genes in the absence of other stimuli (Fig. 4). Since the N-terminal domain of p62 is known to bind the translocation factor p10/NFT2 (Clarkson et al., 1996) and the C-terminal domain binds karyopherin-β (Percipalle et al., 1997) as well as TRAF-3, the effects of over-expressing an N-terminal fragment p62(1-393) ~~p62(1-394)~~ were compared with those of a C-terminal fragment p62(336-522), as well as full-length p62 on RelA-induced NF-κB ~~NF-6B~~ reporter activity. Over-expression of p62(1-393) ~~p62(1-392)~~ inhibits RelA-induced NF-κB ~~NF-6B~~ reporter activity, consistent with an effect of p62(1-393) ~~p62(1-392)~~ on translocation of activated NF-κB ~~NF-6B~~. In contrast, over-expression of p62(336-522) did not alter RelA-induced NF-κB ~~NF-6B~~ reporter activity. The effect of over-expressing

full length p62 was intermediate between p62(1-393) ~~p62(1-392)~~ and p62(336-522). Cells transfected with full-length p62 and p62(1-393) ~~p62(1-392)~~ showed similar morphology and viability to samples transfected with other constructs. In addition, Western blot analysis of lysates used in luciferase assays showed similar levels of RelA expression in p62-, p62(1-393)- ~~p62(1-392)~~-, and p62(336-522) -transfected cells (data not shown). Together, these data indicate that p62(1-393) ~~p62(1-392)~~, and to a lesser extent full-length p62, inhibit nuclear translocation of activated NF- $\kappa$ B ~~NF-6B~~, consistent with a depletion of nuclear translocation factors that bind the N-terminus of p62. In addition, these data indicate that the TRAF-3 binding fragment, p62(336-522), does not measurably alter the translocation of activated NF- $\kappa$ B ~~NF-6B~~ and suggest that this fragment may be used to study effects of p62(336-522) on NF- $\kappa$ B ~~NF-6B~~ activation.

Please replace the paragraph numbered [0139] with the following amended paragraph:

[0139] d. Effects of p62 Fragments on Inducing NF- $\kappa$ B ~~NF-6B~~ Activation

Please replace the paragraph numbered [0140] with the following amended paragraph:

[0140] To study whether the TRAF-3 binding fragment, p62(336-522) regulates NF- $\kappa$ B ~~NF-6B~~ activation, the functional effects of over-expressing p62(336-522) on baseline and CD40-induced NF- $\kappa$ B ~~NF-6B~~ activation were studied in 293T cells. In parallel experiments, p62(1-393) ~~p62(1-392)~~ and

full-length p62 were also studied, although it was expected that their effects on inhibiting the translocation of activated NF- $\kappa$ B ~~NF-6B~~ would inhibit the NF- $\kappa$ B ~~NF-6B~~ reporter assay. In fact, p62(1-393) ~~p62(1-392)~~ and to a lesser extent, full-length p62, inhibit baseline and CD40-induced reporter activity, consistent with their inhibition of NF- $\kappa$ B ~~NF-6B~~ translocation (Fig. 5). In contrast, over-expression of the TRAF-3 binding fragment, P62(336-522) induces approximately 3-fold activation of NF- $\kappa$ B ~~NF-6B~~ reporter gene activity in the absence of CD40 expression (Fig. 5) and approximately 8-fold activation of reporter gene expression when co-transfected with CD40, which is approximately twice the level observed in cells transfected with CD40 and empty pCEP4 expression vector (Fig. 5). Together with the previous finding that p62(336-522) does not affect the translocation of activated NF- $\kappa$ B ~~NF-6B~~ (Fig. 4), these data suggest that p62(336-522), which contains the TRAF-3 binding domain, induces NF- $\kappa$ B ~~NF-6B~~ activation.

Please replace the paragraph numbered [0141] with the following amended paragraph:

[0141] These studies also evaluated the effects of over-expressing full-length TRAF-3 on p62(336-522)-induced NF- $\kappa$ B ~~NF-6B~~ activation. Consistent with previous reports, over-expression of full-length TRAF-3 has little effect on background NF- $\kappa$ B ~~NF-6B~~ reporter gene activity but inhibits the NF- $\kappa$ B ~~NF-6B~~ activation induced by over-expression of CD40 (Fig. 5). Full-length TRAF-3 fails to inhibit p62(336-522)-induced NF- $\kappa$ B ~~NF-6B~~ activation (Fig. 5). In addition, co-expression of full-length TRAF-3 with p62(336-522) and CD40

results in NF- $\kappa$ B ~~NF-6B~~ reporter activity that is approximately equal to the level induced by p62(336-522) alone (Fig. 5). This finding is consistent with the interpretation that full-length TRAF-3 inhibits the component of NF- $\kappa$ B ~~NF-6B~~ activation induced by CD40 over-expression, but not the component induced by p62(336-522) expression. Co-expression of full-length TRAF-3 with p62(1-393) ~~p62(1-392)~~ or full-length p62 resulted in reporter gene activity approximately equivalent to samples expressing either of these p62 constructs alone. Together, these findings suggest that the p62(336-522) effects on inducing NF- $\kappa$ B ~~NF-6B~~ activation are downstream of the inhibitory effects that full-length TRAF-3 exerts on CD40-triggering.

Please replace the paragraph numbered [0142] with the following amended paragraph:

[0142] These studies on the mechanisms by which TRAF-3 mediates signal transduction show that TRAF-3 interacts with p62 nucleoporin and that p62 fragments have distinct effects on NF- $\kappa$ B ~~NF-6B~~ activation and translocation. TRAF-3's ability to bind p62 is not shared by TRAF-2, TRAF-4, TRAF-5, or TRAF-6. The p62 C-terminal coiled-coiled domains were found to mediate interactions with TRAF-3. The p62 C-terminal domain is known also to act as a docking site for import complexes by binding karyopherin- $\beta$  (Rexach and Blobel, 1995; Percipalle et al., 1997) and the N-terminal domain is known to interact with p10/NTF2, an essential factor of the nuclear import machinery (Paschal and Gerace, 1995; Clarkson et al., 1996). To separate these effects, functional studies were performed on N-terminal and C-terminal fragments of p62. Over-

expression of the p62 N-terminal fragment inhibits NF- $\kappa$ B ~~NF-6B~~ activation in a dominant negative manner, consistent with inhibition of nuclear translocation. In contrast, the p62 C-terminal TRAF-3 binding domain induces NF- $\kappa$ B ~~NF-6B~~ activation which suggests a previously unappreciated role for p62 in NF- $\kappa$ B ~~NF-6B~~ activation.

Please replace the paragraph numbered [0143] with the following amended paragraph:

[0143] The role of p62 in NF- $\kappa$ B ~~NF-6B~~ translocation has been inferred from the fact that cytoplasmic NF- $\kappa$ B ~~NF-6B~~ complexes must translocate to the nucleus in order to activate transcription of target genes and that p62 is known to serve as a docking site for karyopherin-complexed transcription factors. The exposed NLS of activated NF- $\kappa$ B ~~NF-6B~~ binds karyopherin- $\alpha$  and this complex binds karyopherin- $\beta$ , which docks the NF- $\kappa$ B ~~NF-6B~~/karyopherin- $\alpha/\beta$  complex to p62 at the nuclear pore (Nadler et al., 1997; Torgerson et al., 1998). In resting cells, NF- $\kappa$ B ~~NF-6B~~ is rendered inactive by interactions with I- $\kappa$ B which masks the NF- $\kappa$ B ~~NF-6B~~ NLS. Signaling by cell surface receptors leads to the dissociation of I- $\kappa$ B and the liberation of NF- $\kappa$ B ~~NF-6B~~ with an exposed NLS (Beg et al., 1992). Over-expression of the NF- $\kappa$ B ~~NF-6B~~ protein RelA bypasses I- $\kappa$ B regulation because free RelA is expressed in excess of cellular I $\kappa$ -B.

Please replace the paragraph numbered [0144] with the following amended paragraph:

[0144] Consequently, over-expressed RelA undergoes nuclear translocation and activates transcription in the absence of exogenous stimuli that normally dissociate I- $\kappa$ B from the pool of inactive NF- $\kappa$ B ~~NF-6B~~. Therefore, the reporter gene activity induced by over-expression of RelA is a measure of p62 mediated nuclear translocation that does not depend on dissociation of I- $\kappa$ B. The finding that over-expression of an N-terminal p62 domain, p62(1-393) ~~p62(1-392)~~, inhibits RelA-induced NF- $\kappa$ B ~~NF-6B~~ reporter gene activity suggests a dominant negative effect by p62(1-393) ~~p62(1-392)~~ on p62-mediated translocation of RelA. Since the N-terminus of p62 binds p10/NTF2 in vitro (Clarkson et al., 1996) and NTF2 is essential for nuclear import (Paschal and Gerace, 1995), it is believed that over-expression of p62(1-393) ~~p62(1-392)~~ in the cytoplasm depletes cellular pools of p10/NTF2, to inhibit translocation of RelA import complexes through the nuclear pore. Thus, these data are consistent with the known requirement for nuclear translocation of RelA in order to exert effects on target gene transcription.

Please replace the paragraph numbered [0145] with the following amended paragraph:

[0145] In contrast ~~contrast~~ to the inhibitory effect of the p62 N-terminal fragment on RelA translocation, over-expression of a C-terminal fragment fails to inhibit RelA translocation. The lack of inhibition by p62(336-522) is surprising because this region is known to be sufficient to bind karyopherin- $\beta$  (Percipalle et al., 1997) and thus might have been expected to inhibit docking of

karyopherin/RelA complexes to endogenous p62 at the nuclear pore, particularly since over expression of the p62 C-terminal domain has been reported to lead to significant accumulation outside the nuclear pore complex (Starr et al., 1990; Carmo-Fonseca et al., 1991). It is unclear why over-expression of p62(336-522) fails to inhibit RelA translocation, but may relate to pore-associated p62 having a higher avidity than cytoplasmic p62(1-393) ~~p62(1-392)~~ for karyopherin- $\beta$ . However, the finding that p62(356-522) does not inhibit nuclear translocation of RelA provided an opportunity to study ~~stud-y~~ its effects on NF- $\kappa$ B ~~NF-6B~~ activation.

Please replace the paragraph numbered [0146] with the following amended paragraph:

[0146] Expression ~~[-expression]~~ of p62(336-522) induces NF- $\kappa$ B ~~NF-6B~~ reporter gene activity in the absence of CD40 signaling and augments the effect of CD40 over-expression on NF- $\kappa$ B ~~NF-6B~~ reporter gene activity. Since over-expression of a p62 C-terminal fusion protein is known to result in expression of substantial amounts of the p62 fusion protein outside the nuclear pore complex in the cytoplasm (Carmo-Fonseca et al., 1991), the ability of p62(336-522) to increase NF- $\kappa$ B ~~NF-6B~~ dependent reporter gene activity arises from triggering the cytoplasmic signaling cascade that liberates activated NF- $\kappa$ B ~~NF-6B~~ from I- $\kappa$ B. The effects of full-length p62 over-expression were intermediate between those of the individual N-terminal ~~N-terminal~~ and C-terminal fragments both on translocation of RelA and on activating NF- $\kappa$ B ~~NF-6B~~. The intermediate effects of full-length p62 are consistent with mixed effects,

in which the p62 C-terminal domain induces NF- $\kappa$ B ~~NF-6B~~ activation and the N-terminal domain inhibits nuclear translocation.

Please replace the paragraph numbered [0147] with the following amended paragraph:

[0147]        The finding ~~finding~~ that the C-terminal p62 fragment induces NF- $\kappa$ B ~~NF-6B~~ activation was unexpected. Since the p62 C-terminal fragment binds TRAF-3, it is interesting to consider how TRAF-3 binding may relate to its effect. Consistent with previous work, full-length TRAF-3 over-expression is known to inhibit NF- $\kappa$ B ~~NF-6B~~ activation triggered by CD40 over-expression (Rothe et al., 1995). Since over-expression of full-length TRAF-3 fails to affect translocation of over-expressed RelA (see Fig.4), full-length TRAF-3 appears to act proximal to nuclear translocation in the receptor-triggered signaling cascade. In addition, full-length TRAF-3 fails to inhibit p62(336-522)-induced NF- $\kappa$ B ~~NF-6B~~ activation, which suggests that the inhibitory effect of full-length TRAF-3 on CD40-induced NF- $\kappa$ B ~~NF-6B~~ activation is also proximal to the inducing effect of p62(336-522). Therefore, over-expression of full-length TRAF-3 may inhibit CD40-induced NF- $\kappa$ B ~~NF-6B~~ activation at the level of TRAF-3 binding to the CD40 cytoplasmic tail, the recruitment of other factors to CD40-bound TRAF-3, or by the formation of homo-trimers of full-length TRAF-3.

Please replace the paragraph numbered [0148] with the following amended paragraph:



[0148] It has ~~{has~~ recently been shown that over-expression of certain TRAF-3 splice-deletion variants induces NF- $\kappa$ B ~~NF-6B~~ activation (van Eyndhoven et al., 1999). In contrast to the effects of over-expressing full-length TRAF-3 alone, co-expression of full-length TRAF-3 with the activating splice-deletion variants augments NF- $\kappa$ B ~~NF-6B~~ activation induced by the splice deletion isoforms alone, suggesting that full-length TRAF-3 stabilizes mixed TRAF-3 trimeric complexes that consist of full-length and splice-deletion TRAF-3 isoforms (van Eyndhoven et al., 1999). Together these observations suggest that TRAF-3 heterotrimers are capable of forming signaling complexes that induce NF- $\kappa$ B ~~NF-6B~~ activation. Therefore, one possible explanation for the NF- $\kappa$ B ~~NF-6B~~ inducing activity of p62(336-522) is that it interacts with such TRAF-3 heterotrimers and activates them. In this regard, TRAF-3 is also known to interact with NIK that plays a role in activating NF- $\kappa$ B ~~NF-6B~~ (24;25). The finding that over-expression of full-length TRAF-3 fails to inhibit p62(336-522) effects, suggests that p62 interactions with TRAF-3 trimers are transient and that each p62 molecule may activate multiple TRAF-3 complexes.

Please replace the paragraph numbered [0149] with the following amended paragraph:

[0149] These considerations ~~{considerations~~ suggest biological roles for TRAF-3:p62 interactions in signaling. By binding TRAF-3, p62 may recruit TRAF-3 signaling complexes to the nuclear pore. Such recruitment would result in local activation of NF- $\kappa$ B ~~NF-6B~~ signaling complexes. In this regard, the NF- $\kappa$ B ~~NF-6B~~ signalosome consisting of IKK and associated molecules has been

shown to contain RelA ~~Re1A~~ (Mercurio et al., 1997; Heilker et al., 1999). Therefore, the local activation of the signalosome (e.g. by TRAF-3 bound NIK) at the nuclear pore complex may liberate activated NF- $\kappa$ B ~~NF-6B~~ in close proximity to the karyopherin- $\beta$  docking site on p62, perhaps facilitating its import. In addition, recruitment of a TRAF-3 signaling complex containing kinases (such as TRAF-3 bound ASK1 (Nishitoh et al., 1998)) to the nuclear pore might result in phosphorylation of p62. Such modification of p62 has been observed (Macaulay et al., 1995; Buss et al., 1994) and correlates with nuclear import of a different transcription factor (Buss et al., 1994). Understanding the roles of TRAF-3:p62 binding and the potential activation of TRAF-3 and p62 are important goals of future research.

Please replace the paragraph numbered [0150] with the following amended paragraph:

[0150]        These data ~~{data~~ may also relate to the essential function that TRAF-3 signaling is known to play in T-dependent antibody production. These observations suggest that the essential roles of TRAF-3 in signaling may be due to its unique ability among TRAF family members to associate with p62. In addition to p62's known roles in mediating nuclear translocation of NF- $\kappa$ B ~~NF-6B~~, the present study suggests that p62:TRAF-3 interactions may be a means by which p62 organizes a signaling complex at the nuclear pore and in which p62 induces NF- $\kappa$ B ~~NF-6B~~ activation. In this regard, certain clinically important anti-inflammatory and immunosuppressive agents, such as acetylsalicylic acid and cyclosporin A, are believed to function by inhibiting steps required for nuclear

translocation of the transcription factors NF-~~κ~~B ~~NF-6B~~ (Yin et al., 1998) and NF-AT (Emmel et al., 1989), respectively. These considerations indicate that TRAF-3:p62 interactions provide a novel target for therapeutic agents that may regulate immune responses.

Please replace the paragraph numbered [0151] with the following amended paragraph:

[0151]        The invention ~~the invention~~ has been illustrated and described with respect to specific illustrative embodiments and modes of practice, it will be apparent to those skilled in the art that various modifications and improvements may be made without departing from the scope and spirit of the invention. Accordingly, the invention is not to be limited by the illustrative embodiments and modes of practice.

Please also delete pages 66-87 of the as-filed specification (paragraphs 205-277 of the published application).